PATENT COOPERATION TREATY

VC

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

GITTO, Serena
Ing. Barzano' & Zanardo Roma S.p.A.
Via Piemonte, 26
I-00187 Roma
ITALIE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.03.2006
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
PCT25791	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IT2004/000689	10.12.2004	11.12.2003

Applicant
TIGEM

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Ladurner, Y

Tel. +49 89 2399-7913

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT25791	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/IT2004/000689	International filing date *(day/month/year)* 10.12.2004	Priority date *(day/month/year)* 11.12.2003
International Patent Classification (IPC) or national classification and IPC INV. A61K31/4985 A61K31/505 A61K31/475 A61K38/12 A61K38/16 G01N33/574 C07K7/00 A61P35/04		
Applicant TIGEM		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand 10.10.2005	Date of completion of this report 29.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bochelen, D Telephone No. +49 89 2399-8150

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2004/000689

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1(b))
   ☐ publication of the international application (under Rule 12.4)
   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-5, 7-58	as originally filed
6	filed with telefax on 10.10.2005

Claims, Numbers

1-32	received on 18.10.2005 with letter of 18.10.2005

Drawings, Sheets

1/19-19/19	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☒ the claims, Nos. 1,5-6
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2004/000689

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-4,11,31
	No:	Claims	1,5-6,10,22
Inventive step (IS)	Yes:	Claims	3-4
	No:	Claims	1-2,5-31
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/IT2004/000689

Re Item I
Basis of the report

1. The amendments filed with the letter dated 18.10.05 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: the restriction to a peptide **having** the amino acid sequence of SEQ ID No 9 in claims 1, 5 and 6. Throughout the original application were mentioned only peptides **comprising** an amino acid sequence of SEQ ID No 9.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. Prior art:

Reference is made to the following documents:

D1: US-B1-6 486 300 (BANDMAN OLGA ET AL) 26 November 2002 (2002-11-26)

D2: COLLIER G R ET AL: "INHIBITION OF LUNG METASTASIS FORMATION BY A RAT OSTEOGENIC SARCOMA SUBCLONE USING PYRIMIDO-PYRIMIDINE DERIVATIVES" AUSTRALIAN AND NEW ZEALAND JOURNAL OF MEDICINE, ROYAL AUSTRALASIAN COLLEGE OF PHYSICIANS, SYDNEY, AU, vol. 15, no. 1, SUPPL 1, February 1985 (1985-02), page 127, XP008046052 ISSN: 0004-8291

D3: BANDO H ET AL: "EFFECTS OF ANTIPLATELET AGENTS ON PULMONARY METASTASES" GANN, JAPANESE CANCER ASSOCIATION, TOKYO, JP, vol. 75, no. 3, March 1984 (1984-03), pages 284-291, XP009013087 ISSN: 0016-450X

D4: BERTRAM J S ET AL: "INHIBITION OF NEOPLASTIC CELL GROWTH BY QUIESCENT CELLS IS MEDIATED BY SERUM CONCENTRATION AND CYCLIC AMP PHOSPHO DI ESTERASE INHIBITORS" JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 18, no. 4, 1982, pages 515-538, XP002329792 ISSN: 0730-2312

D5: NI XIAOHUA ET AL: "Isolation and characterization of a novel human NM23-H1B gene, a different transcript of NM23-H1." JOURNAL OF HUMAN

GENETICS, vol. 48, no. 2, February 2003 (2003-02), pages 96-100, XP002329793 ISSN: 1434-5161

D6: POSTEL EDITH H ET AL: "Mutational analysis of NM23-H2/NDP kinase identifies the structural domains critical to recognition of a c-myc regulatory element" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 93, no. 14, 1996, pages 6892-6897, XP002329794 ISSN: 0027-8424

D7: REYMOND ALEXANDRE ET AL: "Evidence for interaction between human PRUNE and nm23-H1 NDPKinase" ONCOGENE, vol. 18, no. 51, 2 December 1999 (1999-12-02), pages 7244-7252, XP002329795 ISSN: 0950-9232

D8: FORUS ANNE ET AL: "Amplification and overexpression of PRUNE in human sarcomas and breast carcinomas: A possible mechanism for altering the nm23-H1 activity" ONCOGENE, vol. 20, no. 47, 18 October 2001 (2001-10-18), pages 6881-6890, XP002329796 ISSN: 0950-9232

D9: ZOLLO M ET AL: "Prune and nm23-H1 and nm-23 H2 (NDP-Kinase) proteins: Involvement in cancer" AMERICAN JOURNAL OF HUMAN GENETICS, vol. 69, no. 4 Supplement, October 2001 (2001-10), page 273, XP009047948 & 51ST ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; SAN DIEGO, CALIFORNIA, USA; OCTOBER 12-16, 2001 ISSN: 0002-9297

D10: DATABASE Geneseq [Online] 26 June 2001 (2001-06-26), "Human cDNA clone (5'-primer) SEQ ID NO:5290." XP002329797 retrieved from EBI accession no. GSN:AAH08455 Database accession no. AAH08455

D11: DATABASE Geneseq [Online] 6 November 2003 (2003-11-06), "Human intracellular signalling molecule INTSIG-44, SEQ ID NO:44." XP002329798 retrieved from EBI accession no. GSN:ADA13362 Database accession no. ADA13362

D12: DANGELO A ET AL: "The human cyclic nucleotides phosphodiesterase (PDE) Prune protein: A dual cellular compartment localization and functional properties." AMERICAN JOURNAL OF HUMAN GENETICS, vol. 71, no. 4 Supplement, October 2002 (2002-10), page 513, XP002329885 & 52ND ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; BALTIMORE, MD, USA; OCTOBER 15-19, 2002 ISSN: 0002-9297

If not indicated otherwise the relevant passages are those mentioned in the

search report.

Document D1 discloses the use of human nm23, comprising a peptide sequence of SEQ ID No 9 of the application, for inhibiting metastasis.

Document D2 discloses the use of dipyridamole for inhibiting metastasis.

Document D3 discloses the inhibition of metastasis by dipyridamole

Document D4 discloses the inhibition of metastasis of Lewis lung carcinoma by the PDE inhibitor isobutyl-methylxanthine.

Document D5 discloses the sequence of nm23-H2, defined as a putative metastasis suppressor, which comprises a peptide of sequence of SEQ ID No 9 of the application.

Document D6 discloses the sequence of nm23, comprising an amino acid sequence of SEQ ID No 9 of the application, which is a presumed regulator of tumour metastasis.

Document D7 discloses that Prune interacts with nm23 and the uncoupling of this interaction might lead to neuroblastoma progression.

Document D8 discloses the over-expression and amplification of PRUNE assessed by immunohistochemistry, FISH and northern blot in tumours expressing nm23 and in metastasising tumours.

Document D9 discloses the interaction of the PDE Prune with the tumour metastasis inhibitor gene nm23-H1. Document D9 discloses that Prune is amplified in tumour cells as shown by FISH and immunohistochemistry.

Document D10 discloses a nucleic acid sequence comprising the sequence of SEQ ID No 1 of the application which is a 5'-primer.

Document D11 discloses a peptide comprising a sequence of SEQ ID No 4 of the application and antibodies specific for this peptide.

Document D12 discloses that the Prune protein possesses phosphodiesterase activity.

2. Novelty (Art. 33 (1) and (2) PCT):

2.1 Claim 1 is not novel over the disclosure of documents D1. Claim 1 is interpreted as relating to a peptide **comprising** a sequence of SED ID No 9 (see above point 1). Document D1 does not disclose that nm23, which comprises the amino acid sequence of SEQ ID No 9, is an inhibitor of the cyclic nucleotide phosphodiesterase activity of Prune, however D1 discloses the inhibition of metastasis by these peptides. The presence of a mechanism of action described in the application, i.e inhibition of Prune activity, cannot be used to delimit the present claims from the state of the art. The end effect of the presently claimed invention is the treatment of metastasis using the same peptide as disclosed in the prior art. The mechanism of action is therefore merely a discovery of how the peptide comprising the amino acid sequence of SEQ ID No 9 could work. Claim 1 does not fulfill the requirements of Art. 33(2) PCT.

2.2 Claims 5 and 6 are interpreted as relating to a peptide **comprising** a sequence of SED ID No 9 (see above point 1). Said claim is lacks thus novelty over D1 and D5-D6.

2.3 Claim 10 lacks novelty over document D8-D9 which discloses the increased expression of Prune in metastasising tumours (see p6882 col2 1st §). Claim 11 does not fulfill the requirements of Art. 33(2) PCT.

2.4 Claim 22 lacks novelty over documents D7-D9 which disclose the detection of PRUNE by FISH (D7: page 7246 col 1 §1; D8: p6887 col2; D9: abstract).

3. Inventive step (Art. 33 (1) and (3) PCT):

3.1 The peptide comprising a sequence of SEQ ID No 10 which is subject-matter of claims 3-4 is neither disclosed nor suggested in the prior art. Claims 3-4 fulfill the requirement of Art. 33(3) PCT. The use thereof for preventing metastasis would involve an inventive step.

3.2 Document D12 discloses that the Prune protein possesses phosphodiesterase

catalytic activity. The method of screening of claim 7 uses a specific cell line overexpressing h-PRUNE. However, it would be obvious for a skilled man to use a cell line overexpressing h-PRUNE in a method for screening inhibitors of phosphodiesterase activity. Furthermore, the use of the specific cell line of claim 7 does not result in an unexpected advantage over the prior art. Claim 7 does thus not fulfill the requirement of Art. 33(3) PCT.

3.3 Claim 11 differs from document D8 (see page 6888 col2 1st§) in that a monoclonal antibody is used. However, it would be obvious for a skilled to use a monoclonal antibody against PRUNE instead of a polyclonal antibody. Claim 11 does thus not fulfill the requirement of Art. 33(3) PCT.

3.4 Antibodies directed to Prune are known in the art (see D8: p6885 fig3). It would be obvious for a skilled man to produce an alternative monoclonal antibody specific for Prune. Claim 32 thus lacks inventive step in the sense of Art. 33(3) PCT.

3.5 Dependent claims 8-9, 13-21 and 23-31 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step for the following reasons:

Claim 2 is a selection of specific tumours which is not inventive in view of D1.

Claims 8-9 relate to specific conditions of screening which would be obvious in view of D12.

Claims 12-14 are obvious in view of D8. The use of an alternative monoclonal antibody is not inventive.

The use of specific primers or labelling in the methods of claims 12-21 and in the kit of claims 23-31 cannot be considered as involving an inventive step.

3.6 It is further noted that an inventive step would be acknowledged for the use according to claim 1 of IC261.

NIIHGSDSVESAEKEGGGYGRKKRRQRRR (SEQ ID No 10); and characterised in that it is permeable because of the sequence GGGYGRKKRRQRRR.

It is a further object of the present invention a screening method for h-PRUNE-inhibiting compounds, comprising the following steps:

a) selection of at least one phosphoesterase (PDE) inhibiting compound or derivative, structural analogue or isomer thereof;

b) administration of said at least one compound at concentration between 0.05 µM and 10 µM to one h-PRUNE overexpressing cellular line;

c) quantitative analysis of the cyclic nucleotide phosphodiesterase activity of h-PRUNE and/or analysis of the cell motility versus the concentration of said at least one compound and the chemo-attractant and selection of the compound able to inhibit said phosphodiesterase activity between values from 0.01 to 1 $pmol/min^{-1}/ug^{-1}$ and/or inhibit said motility up values between 200 and 1200 cells.

The inhibition of the cyclic nucleotide phosphodiesterase activity of h-PRUNE by a compound tested as inhibitor of said activity can be established by evaluation of IC_{50} for the aforesaid compound.

The cell line overexpressing h-PRUNE is the following:

MDA-C100 435 prune #4 (deposited at CBA in Genoa on 10/12/2004).

Quantitative analysis of the cyclic nucleotide phosphodiesterase activity of h-PRUNE can be performed by hydrolysis tests of the c-AMP and/or c-GMP substrates. Said substrate is used at concentration between 0,008 µM and 1 µM.

It is a further object of the present invention a method for the preparation of a pharmaceutical composition comprising the method as above described involving the additional step d) of mixing of at least an identified compound, or derivative, structural analogue or isomer thereof, along with one or more coadjuvants and/or pharmacologically acceptable excipients.

Furthermore the present invention pertains to the use of h-PRUNE inhibiting compounds selected according to the method as above defined, for the preparation of a medicament for prevention and treatment of metastases of tumours characterised by an overexpression of h-PRUNE, wherein said tumours can be breast carcinomas, sarcomas, neuroblastomas and melanomas.

Further object of the present invention is a method for detection of h-PRUNE in a biological sample for diagnosis of metastases of the tu-

CLAIMS

1.Use of inhibitors of h-Prune cyclic nucleotide phosphodiesterase activity for the preparation of a medicament for prevention and treatment of tumour metastases characterised by an overexpression of h-PRUNE, said inhibitors being selected from the group consisting of a peptide having the following amino acidic sequence NIIHGSDSVESAEKE (SEQ ID No 9); a peptide comprising the following amino acidic sequence NIIHGSDSVESAEKE GGGYGRKKRRQRRR (SEQ ID No 10); vinpocetine, IC261 and derivatives, structural analogues and isomers thereof.

2.Use according to claim 1, wherein tumours characterised by an overexpression of h-PRUNE are breast carcinoma, sarcoma, neuroblastoma, prostate tumour, pancreatic tumour, colon carcinoma tumour, rectal tumour, medulloblastoma, epitelioma, epatocarcinoma, cell T or cell B lymphomas, myeloma and melanoma, and pulmonary tumour.

3.Peptide comprising the following amino acidic sequence: NIIHGSDSVESAEKEGGGYGRKKRRQRRR (SEQ ID No 10) characterised in that it is permeable.

4.Peptide comprising the following amino acidic sequence: NIIHGSDSVESAEKE GGGYGRKKRRQRRR (SEQ ID No 10) and characterised in that it is permeable and it is an inhibitor of h-Prune cyclic nucleotide phosphodiesterase activity, for use in medical field.

5.Peptide having the following amino acidic sequence: NIIHGSDSVESAEKE (SEQ ID No 9).

6.Peptide having the following amino acidic sequence: NIIHGSDSVESAEKE (SEQ ID No 9) characterised in that it is an inhibitor of h-Prune cyclic nucleotide phosphodiesterase activity, for use in medical field.

7.Screening method for h-PRUNE-inhibiting compounds, comprising the following phases:

a) selection of at least a phosphoesterase (PDE) inhibiting compound or derivative, structural analogue or isomer thereof;

b) administration of said at least one compound at concentration between 0,05 μM and 10 μM in a cell line overexpressing h-PRUNE, wherein said cellular line is MDA-C100 435 prune #4;

c) quantitative analysis of the cyclic nucleotide phosphodiesterase activity of h-PRUNE and/or analysis of cellular motility versus concentration of said at least one compound and chemo-attractant and

selection of compound able to inhibit said phosphodiesterase activity between the values from 0.01 to 1 pmol/min^{-1}/ug^{-1} and/or inhibit said motility up to the attainment of the values between 200 and 1200 cells.

8.Screening method according to claim 7, wherein the quantitative analysis of step c) is carried out by hydrolysis tests of the c-AMP and/or c-GMP substrate.

9.Screening method according to claim 7, wherein the substrate is used at concentration between 0,008 μM and 1 μM.

10.Method for in vitro detection of h-PRUNE in a biological sample for metastases diagnosis of tumours characterised by an h-PRUNE overexpression by immunological assay, FISH analysis, Real-time PCR, in situ hybridization.

11.Method for in vitro detection of h-PRUNE according to claim 10, comprising the following steps:

a) bring into contact said biological sample with at least one anti-h-PRUNE monoclonal antibody;

b) detection of the antigen-antibody complex;

c) quantitative analysis of the antigen-antibody complex.

12.Method according to claim 11, wherein said biological sample is a tissue section or biological fluid.

13.Method according to any one of claims from 10 to 12, wherein said anti-h-PRUNE antibody is the monoclonal antibody able to recognise and bind selectively the h-PRUNE recombinant protein, characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004)

14.Method according to any one of claims from 10 to 13, wherein said anti-h-PRUNE antibody is labelled with a radioisotope, fluorescent molecule or enzyme.

15.Method for in vitro detection of h-PRUNE according to claim 11, wherein said detection and quantitative analysis of the antigen-antibody complex are performed by immunohistochemistry, immunoprecipitation, immunofluorescence, ELISA, immunoblotting analyses.

16.Method according to claim 10, wherein PCR Real time primers specific for h-PRUNE comprise the sequences:

5'-AGAGATCTTGGACAGGCAAACT-3' (SEQ ID No 1);

3'-CCATGTTGACACAGTCCAGGAT-5' (SEQ ID No 2);

or their complementary sequences.

17.Method according to claim 10, wherein the labelled probe for Real-time PCR or in situ hybridization comprise the oligonucleotidic sequence: CTGCATGGAACCATC (SEQ ID No 3) or its complementary sequence or the sequence wherein T is replaced by U.

18.Method according to claim 17, wherein said labelled probe for Real-time PCR is linear or circular one.

19.Method according to any one of claims 17 and 18, wherein said probe is labelled with at least one radioisotope and/or fluorochrome.

20.Method according to any one of claims from 17 to 19, wherein said probe is labelled with at least a fluorochrome at 5' and/or 3'.

21.Method according to any one of claims from 17 to 20, wherein said fluorochrome is 6-carboxifluorescein.

22.Diagnostic kit for the detection of h-PRUNE in a biological sample for metastases diagnosis of tumours characterised by an h-PRUNE overexpression comprising at least one anti-h-PRUNE monoclonal antibody, or a pair of primers specific for h-PRUNE or labelled oligonucleotidic probe specific for h-PRUNE.

23.Diagnostic kit according to claim 22, wherein the tumours characterised by an h-PRUNE overexpression are breast carcinoma, sarcoma, neuroblastoma, melanoma.

24.Diagnostic kit according to any one of claims 22 and 23, wherein said anti-h-PRUNE antibody is characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).

25.Diagnostic kit according to claim 24, wherein said anti-h-PRUNE monoclonal antibody is labelled with a radioisotope, fluorescent molecule or enzyme.

26.Diagnostic kit according to claim 22, wherein said pair of primers specific for h-PRUNE comprises the sequences:

5'-AGAGATCTTGGACAGGCAAACT-3' (SEQ ID No 1);

3'-CCATGTTGACACAGTCCAGGAT-5' (SEQ ID No 2);

or their complementary sequences.

27.Diagnostic kit according to claim 22, wherein said labelled oligonucleotidic probe for Real-time PCR or in situ hybridization comprises the oligonucleotidic sequence:

CTGCATGGAACCATC (SEQ ID No 3)

or its complementary sequence or the sequence wherein T is replaced by U.

28.Diagnostic kit according to claim 27, wherein said labelled oligonucleotidic probe for Real-time PCR is linear or circular one.

29.Diagnostic kit according to any one of claims 27 and 28, wherein said oligonucleotidic probe is labelled with at least one radioisotope and/or fluorochrome.

30. Diagnostic kit according to any one of claims from 27 to 29, wherein said probe is labelled with at least one fluorochrome at 5' and/or 3'.

31.Diagnostic kit according to claim 30, wherein the fluorochrome is 6-carboxifluorescein.

32. Monoclonal murine antibody able to recognise and bind selectively the h-PRUNE recombinant protein, characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).